STUDIES ON THE PHOSPHORESCENCE OF GELATINE AND FLUORESCEIN AT LOW TEMPERATURES.

By Kazuo OCHIAI.

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It is already known that many organic compounds are phosphorescent at low temperature, first discovered by Dewar[1] and further studied by Nichols, Meritt[2] and Kowalski[3] and others.[4] Chemically pure substances show, generally, after-glow of very short duration at low temperature, while they show after-glow of rather long duration (say more than 1 sec.) when dissolved or suspended in a suitable medium and are exited by a ray of suitable wave-length at suitable temperature. Gelatine itself shows brilliant phosphorescence at the temperature of liquid air as shown by Dewar. Moreover, gelatine prolongs duration of after-glow of some substances mixed with it. Though it is not sure whether " pure " gelatine is phosphorescent or not, its phosphorescence depends largely upon the temperature and the water content.

It was also known that fluorescein is phosphorescent when it is dissolved (or suspended) in boric-[5] tartaric-[6] and citric acid.

Experiments on the phosphorescence of gelatine are described in the first part of this paper, and of fluorescein in various mediums are described in the second part.

Part 1. Phosphorescence of Gelatine. As gelatine is phosphorescent only at low temperature, it was sealed in a small test-tube and laid in a cryostat during the experiment. The cryostat was composed of a Dewar's vessel containing pentane or petroleum ether and cooled by a regulating flow of liquid air in a spiral tube within the vessel. A 100 volt, 250 watt incandescent lamp was used as the light source and the light was condenced and filtered by a flask containing ca. 1% cupric sulphate solution before entering the Dewar's vessel.

Time duration of after-glow, denoted by t in the following, was determined subjectively, the accuracy of which was ca. 1 second even when the eyes were rested fully in the darkness.

(1) Dewar, " Collected Papers ", Vol. I (1927), p. 409.
(2) Kayser, " Handbuch der Spektroskopie ", Vol. IV, p. 1027.
(3) Kowalski, *Physik. Z.*, **12** (1911), 956.
(4) For instance, Pringsheim, " Fluorescenz und Phosphorescenz ", 3ed. (1928).
(5) E. Tiede and P. Wuff, *Ber.*, **55** (1922), 588; R. Tomaschek, *Ann. Physik*, **76** (1922), 612.
(6) B. Batscha, *Ber.*, **58** (1925), 187.

Nelson's No. 2 Photographic Gelatine was used in the experiments. Gel was prepared in the following manner: gelatine was swelled for about an hour in distilled water, and then warmed to 30–40°C. until it dissolve into a sol. Now one part was sealed off in a small test-tube for using in the experiment, while another part was dried at 110°C. in an air bath to determine the composition of the gel. A gel thus dried was noted as 100% gelatine. The composition of the ordinary gelatine was found to be 82%. It coloured slightly yellow and contained ca. 3.5% ash.

When gel of 15 to 12% gelatine was frozen, test-tube was broken frequently, probably due to expansion of gel as it frozen. If carefully cooled from the bottom of tube, breakage might be got rid off, but even if bear surface of a gel came in contact with the liquid of the bath (pentane or petroleum ether) no change in t was observed.

Colour of the phosphorescence is white, but in cases of 82% and 100% gels, bluish green colour appeares distinctly. Change of colour in the course of after-glow cannot be observed definitely.

Before measuring t, we must know its relation to the time of excitation. General behaviour is shown in Fig. 1. Thus if excited longer than 5 seconds a constant value of t is obtained. Excitation more than 5 minutes showed no change of the value of t. In the following experiments, therefore, the time of excitation was always 5 to 10 seconds. The results of the experiments are shown in Table 1 and Fig. 2. The hysteresis of these after-glow duration, t, is small and if the sample be kept 10 minutes or more in the cryostat t-value becomes quite constant.

Table 1.

T=temperature in absolute scale. t=duration of after-glow in seconds.

100%		82%		58.5%		35%	
T	t	T	t	T	t	T	t
290	12	265	8	233	6	225	3
265	15	260	13	213	18	220	6
244	21	242	17	203	27	213	15
233	23	235	20	193	30	205	20
213	26	219	25	183	32	190	30
203	27	205	30	173	34	170	34
175	27	185	33			146	34
158	29	173	30			128	32
131	30	136	26			84	35
123	31	123	25				
84	35	84	35				

18.6%		4%		0.8%		0.04%	
T	t	T	t	T	t	T	t
213	13	213	5	204	2		
203	19	203	7	183	27	203	7
193	24	188	27	174	29	193	12
183	29	174	30	166	29	183	15
173	31	166	33	155	29	173	22
		159	34	152	32		
		140	34	135	29		
		131	32				
		84	34				

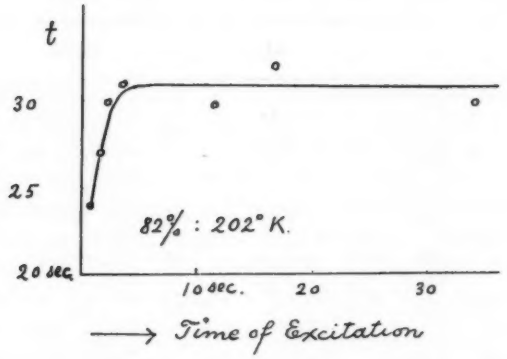
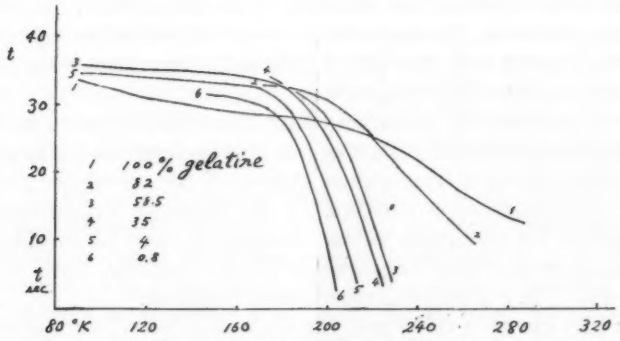


Fig. 1



Temp. in absolute scale.

Fig. 2

The t-value of the gels containing less than 20% gelatine varies markedly by the heat treatment of them. It was observed that a dilute gel (gelatine content less than 20%) is heated for several hours at 80° to 90°C. after the preparation as described before, the value of t changes markedly. The gel was again melted and heated, then again different t-value will be obtained. This cannot be explained as a result of change in composition due to evaporation. Gel of initial composition 20% showed hardly the heating effect.

Some examples of t-values when excited by a 100 volt, 1000 watt lamp are shown in Table 2.

Table 2.

Excited by a 1000 watt lamp.

T	35%	82%	100%
250	—	15	22
233	—	26	29
220	15	—	—
213	—	—	33
195	32	—	34
183	—	41	—
155	46	—	—
150	—	36	30

It is clear from Fig. 2, etc. that, as a rule, t suddenly increases at about 220°K., and further cooling has no remarkable effect on it. Moreover, as concentration increases, the temperature where it begins to be phosphorescent becomes higher and, besides, t gradually decreases from its nearly constant value as the temperature rises.

Then, to answer the question about the relation between phosphorescence and composition of gels, it seems best to compare the temperature and the inclination of t-temperature curves of Fig. 2 where t increases suddenly. As a rough epproximation we may take the temperature where t takes a half of its maximum value. Because it may be considered that these temperature is rather independent of the quantity, form of surface, etc. of the gel. Fig. 3 shows the results thus obtained.

Frozen gels can be divided into three classes from their appearances: gels containing 4 to 5% or less geletine separates laminae of ice and gelatine alternately which reminds of Liesegang's ring, more concentrated but less

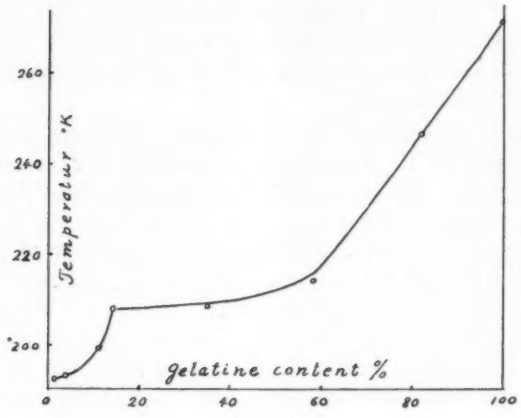


Fig. 3.

than about 60% gelatine freeze to a white solid probably due to irregularly separated small flocks of ice, and more concentrated gels retain their appearance at the room temperature even in the liquid air.

Returning to the present problem, we see here also analogous phenomena. Nearly horizontal part in the middle of the curve in Fig. 3 may be attributed to the separation of gelatine of constant structure, say identical degree of hydration, etc. The inclination in the right side of the curve may be explained as the change in structure, say degree of hydration decreases and thus disturbance of water molecule to the mechanism of phosphorescence is removed. Rather sharp "Knick" in the left side of the curve may be undue, but it is probable that in this region the structure of the gel is distinctly different from the gels containing more than 15% gelatine. Remarkable heating effect may partly due to chemical change like hydrolysis and partly due to the change of degree of dispersion of hydrated gelatine. Of cource the extreme left side of the curve must fall to very low temperature because distilled water is not phosphorescent even in the liquid air in the region of wave length of this experiment.

Part 2. Phosphorescence of Fluorescein. It is well known that fluorescein is phosphorescent when it is buried in boric-, tartaric- and citric acid, and grape sugar containing several organic acids in it. Fluorescein may be dissolved in these substances or in a state of colloidal suspension but we are not sure to which it belongs. B. Batscha[1] has suggested that fluorescein is phosphorescent only when substances of acidic nature co-

(1) Loc. cit.

exists with it. The writer found that fluorescein is also phosphorescent when it is buried in sulphuric acid, hydrogen chloride, nitric-, formic, acetic-, butylic-, oxalic-, and succinic acid at low temperatures. All these acids give coloured solid matter.

Experiment is described in the followings. Exciting light is the same which was used in Part 1. Fluorescein is dissolved in concentrated sulphuric acid considerably. The solution shows slight surface fluorescence and is light to dark orange in colour and when cooled below −37°C. it freezes to yellow silky crystal or brown glassy mass as the quantity of fluorescein increases. 0.005, 0.05 and 2.6 mol/litre solutions of fluorescein were frozen and found to be intensively phosphorescent. Colour and duration of afterglow was nearly the same for them all. Duration was about 15 seconds at −37°C., but if it is cooled below −100°C., then after the cease of orange yellow intense phosphorescence, very weak phosphorescence lasts more than 3 minutes.

Next, a small quantity of sulphuric acid is added to ca. 0.0001 mol/litre aqueous fluorescein solution. On freezing this solution a brilliant phosphorescence was appeared. To the same 0.0001 mol/litre solution hydrochloric-, nitric-, acetic-, formic-, and butylic acid were added respectively and again found to be phosphorescent below their freezing temperature, Fluorescein is soluble in liquid hydrogen chloride to a small quantity and by cooling it gives yellow transparent solid. This is also phosphorescent but the colour of it is rather yellowish.

It was known that fuming nitric acid gives tetra-nitro-fluorescein. This substance was prepared and dissolved in water and frozen, but the result was negative, showing that the substitution cannot be the cause of phosphorescence.

Oxalic- and succinic acid containing fluorescein showed very weak phosphorescence even at the temperature of liquid air in contrast with the result of Batscha, where oxalic acid gave rather intense phosphorescence (in grape sugar). Molten succinic acid dissolves fluorescein and gives coloured crystal but showed none.

Some salts of the above mentioned acids like KCl, $CaCl_2$, $Ca(CH_3CO_2)_2$, etc. and some alkalies like NH_4OH, NaOH, KOH, $Ba(OH)_2$ were added to the aqueous fluorescein solution and frozen, but the result was rather negative, neverthless some of them seem to show phosphorescence of short duration. The same for picric acid, acid potassium phthalate, benzoic acid and glycerine.

Substances like acids probably go into complex molecule (even if lablie as a complex compound) with fluorescein, and in this complex molecule the

positive part of the polar group is directed to fluorescein. In a complex molecule we understand that molecules of acid and fluorescein are situated so near that the polar group serves nearly as a positive charge to fluorescein. In the interior of this complex molecule the mechanism analogous to that of "crystalo-phosphore"[1] may occur and the phosphorescence is observed. As formerly described NaCl, etc. show no phosphorescence with fluorescein. This may partly due to spare solubility of fluorescein in them or the formation of complex molecule is difficult, but even these conditions are fulfilled large ionic volume of sodium, etc. compared with that of hydrogen ion may give a negative result. Further the structure of the fluorescein in the phosphorescent state should be akin to that in the ionized state in liquid solution, and the spectra of phosphorescence may be in the same system of that of fluorescence, because the positive part of the polar group situated so nearly as supposed above will force fluorescein to take analogous structure as in a ionized state.

In conclusion the writer wishes to express his cordial thanks to Prof. J. Sameshima for his kind guidance throughout this experiment.

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BIOCHEMICAL STUDIES ON RICE STARCH. V. COMPARATIVE STUDIES ON RICE, BOILED RICE AND MOCHI.

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In our daily life, in the Orient, it is our custom to eat rice at every meal, the best of food, after it is boiled with water, or steamed and pounded into a cake called mochi (usually prepared from glutinous rice). Mochi is said to be much more digestible for the digestive organs than even boiled rice. Therefore, the chemical changes of the starch of rice in the process of boiling with water, and also in the trituration process of making mochi aroused our interest, and induced us to investigate the subject from the point of view of bio-chemistry, and the following experiments were undertaken.

Preparation of Materials and Their Analysis. 1. *Boiled rice*: The rice named Asahi, which was used in the previous experiments,[2] polished, and

(1) R. Tomaschek, *Ann. Physik*, **76** (1922), 612.
(2) This Bulletin, **5** (1930), 64, 69, 87 & 91.

boiled with water in the following quantities: 500 gr. of polished rice boiled with 750 c.c. of water at 105°C. for 25 min., then, taken off the fire but otherwise untouched left for about 10 min.

2. *Mochi*: 500 gr. of polished rice, washed and soaked in water over night, and steamed for 45 min. at 99°–110°C., triturated with a wooden pestle to make a sticky mass with the addition of a little water, and no more individual rice granules are to be seen.

In analysis, the boiled rice and mochi were soaked in cold acetone and let stand in a cold place for 3 days, filtered, and the filtrate was evaporated to dryness and the fat remaining in the residue was extracted with petroleum ether. With the residual substance the content of reducing sugars was determined as usual.

The insoluble residue in acetone was dried, ground into powder in a stone mill, treated with water at room temperature for a few hours, and filtered. The content of reducing sugars in the filtrates was estimated before and after the hydrolysis with 3 % H_2SO_4 as usual. The starch-content in the final residue was determined after it was hydrolysed with the mineral acid. The results are shown in the following table with the data for polished rice in comparison.

	Rice		Mochi	Seedlings
	Polished	Boiled		
H_2O	13.6%	62%	55%	—%
Starch	76	67	68	56
Red. sugars	0	0.7	0.7	4
Sol. polysacch.	0	9	9	10

As may be seen from this table, the polished rice contained no reducing sugar or soluble polysaccharides, but they were found in boiled rice and mochi, and their formation was assumed to occur from the hydrolysis of starch. When the chemical changes which happen to the rice starch during cooking are compared with those in the germination of seeds, it may be noticed that hydrolysis of starch occurs in both cases, but the complete decomposition of the sugar molecule into CO_2 and H_2O, is to be found only in the germination.

To learn the difference in digestibility of two kinds of cooked rice—boiled rice and mochi—which would arise due to the differentiation of their physical state, the action of diastase and saliva under various P_H on the freshly prepared boiled rice and mochi was investigated. In the experi-

ment, they were ground in a mortar and suspended in water, and 100 gr. of the sample, 500 c.c. of water, 4 gr. of Taka-diastase, and 20 c.c. of the writer's saliva were mixed separately. The mixtures were allowed to stand at 35°C. for 3 hours, and the colour test with iodine, and determination of the content of soluble sugars by means of Fehling's solution were made, and the results are shown in the table below.

	Boiled rice		Mochi	
	Diastase	Saliva	Diastase	Saliva
Rotatory power	19	15	23	19
Red. sugars	17	12	12	15
Sol. polysacch.	26	17	45	15

	Rice starch	
	Rice diastase	Saliva
Red. sugars	25	38
Sol. polysacch.	25	39
Starch	46	20

As will be seen in the table, the conversion of rice starch by saliva into soluble sugars is greater than that by rice diastase, but the reaction to starch of boiled rice and of mochi is reciprocated with diastase and saliva in each case. In any way, starch in mochi seems easy to convert into soluble sugars by means of the hydrolytic enzyme in diastase and saliva.

In order to learn more precisely the chemical nature of the reaction products which were produced by the action of diastase and of saliva from boiled rice and from mochi, the water soluble substance separated from the reaction products was treated with basic lead acetate, and filtered, H_2S gas was passed to remove the lead in the solution, and the filtrate was concentrated into a small volume. Alcohol was added to make 80% of an alcohol solution. The first precipitate thus formed was separated, and the filtrate was concentrated and the second precipitate was formed with 90% of alcohol. Finally, the filtrate was evaporated to a thick syrup and treated with absolute alcohol to form the third precipitate. Of these precipitates, the rotatory power and reducing power of the Fehling solution were deter-

mined, and confirmed for the occurrence of glucose and maltose in the products by the osazone test. The results are shown in the following table.

Boiled rice

	Diastase				Saliva			
	Yields %	$[\alpha]_D$	R.p.	Osazone test	Yields %	$[\alpha]_D$	R.p.	Osazone test
A	26	136	32	M	17	168	30	M
B	11	109	52	M	—	—	—	
C	6	107	58	M+G	12	138	40	M

Mochi

	Diastase				Saliva			
	Yield %	$[\alpha]_D$	R.p.	Osazone test	Yield %	$[\alpha]_D$	R.p.	Osazone test
A	45	145	38	M	15	169	26	M
C	12	112	56	M+G	15	139	47	M

where: A=80 % alcohol precipitate,
B=90 % ,, ,,
C=absolute ,, ,,
M=moltosazone,
G=glucosazone,
R.p.=Reducing power indicated as the percentage of d-glucose.

By the action of diastase on boiled rice and mochi, maltose as well as glucose were produced, but with saliva only maltose was noticed to form.

In mochi, the reaction product consisted mostly of soluble polysaccharides, and the production of reducing sugars and soluble polysaccharides from boiled rice by diastase and saliva is proportionately similar.

The action of diastase and saliva on boiled rice and mochi seems to differ in their reaction products from each other due to the differentiation of the physical state of these two preparations.

Of these reaction products, the distribution of dextrin, maltose and glucose were also determined by Wiley's method[1]; the reducing sugars in

(1) Wiley, "Agricultural Analysis" (1897) Vol. III, p. 288.

the products are removed by fermentation with yeast, and dextrin and maltose were determined separately. The results are as follows:

	Boiled rice		Mochi	
	By diastase	By saliva	By diastase	By saliva
Dextrin	37	38	35	37
Maltose	30	62	46	63
Glucose	23	–	12	–

As will be seen in this table, when saliva acted on both boiled rice and mochi, maltose and dextrin were formed, and with diastase some glucose was noticed as a reaction product. Mochi is easily converted by diastase into maltose; but in boiled rice conversion of the starch into simpler sugar takes place.

When foods with saliva come into the stomach their digestion was said to proceed in an acidic medium of the gastric juice which usually shows $P_H=1.7$, and therefore, the chemical changes in the constituents of boiled rice and of mochi by diastase and by saliva were investigated under conditions similar to the fluid of the digestion organ, namely at $P_H=1.7$, using KCl—HCl for buffer. The results are shown in the following table.

	Diastase		Saliva	
	$P_H=1.7-2.3$	$P_H=7.8$	$P_H=1.7$	$P_H=6.2-6.5$
Mochi	1.6	23	0.9	19
Boiled rice	1.1	19	0.8	15
Starch	–	50	0.4	28

The numbers indicate the percentage of reducing sugars calculated as d-glucose.

The action of diastase and saliva on the starch of boiled rice and mochi proceeded in a little acid side, $P_H=6\sim7$ to form simpler sugars but no action was observed in an acidic medium such as $P_H=1.7$.

Discussion. As a whole, the action of water on starch at 105°C. will result to produce soluble sugars by hydrolysis, and the beating of rice starch with a wooden pestle in presence of water will also promote in some

degree the hydrolysis of the starch, converting it into soluble polysaccharides and reducing sugars. When diastase or saliva acts on mochi and boiled rice, the formation of soluble polysaccharides and reducing sugars is much more intensive. The chemical change of the starch in rice by cooking with boiled water and trituration resembles, in some respects, the germination of rice seeds; the difference is that the velocity in the conversion of starch into soluble sugars is greater in the former, but the conversion of starch into carbon dioxide and water takes place in the latter.

When comparing the action of amylase which occurred in diastase or saliva in boiled rice and mochi, the latter is more affected by the enzyme.

In closing, the writer would like to offer her hearty thanks to Professor S. Komatsu of Kyoto Imperial University, for the experiments were performed under his kind direction and never failing encouragement.

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THE ACTION OF PERBENZOIC ACID ON GLUCAL AND ITS DERIVATIVES.

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M. Bergmann[1] has already observed that when the glucal derived from glucose was oxidised with perbenzoic acid in acetic acid ethyl ester, it was almost completely converted into mannose and thus he discovered a new way for the transformation of glucose into mannose, and such a conversion of sugar was previously regarded as being effected by intramolecular conversion in an aqueous alkali solution.[2] The mechanism of the new transformation was explained by assuming a transitory formation of 1–2 anhydromannose from glucal, which will then be resolved by taking one molecule of water into the mannose.

The present writer has undertaken the experiment in order to study whether the conversion of glucal into mannose will take place not only in glucal but also in its derivatives, such as triacetyl glucal, and diacetyl pseudo-glucal or not, by oxidation with perbenzoic acid in a chloroform solution.

(1) Bergmann & Schotte, *Ber.*, **54** (1921), 440.
(2) Lobry de Bruyn & van Ekenstein., *Rec. Trav. Chim.*, **14** (1895), 1010-1023.

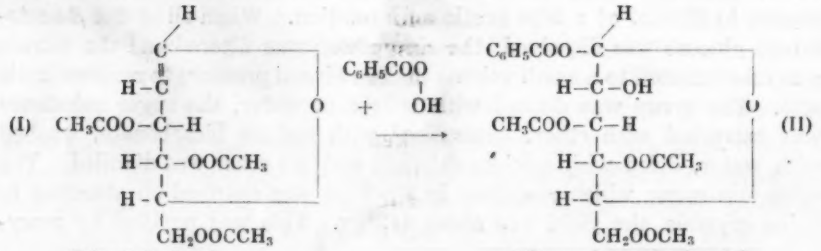
Glucal as Bergmann has stated, was oxidised instantly by perbenzoic acid, and converted into mannose. While, the oxidation of triacetyl glucal with perbenzoic acid was very slow, taking about four days, and diacetyl pseudo-glucal was noticed to undergo in oxidation with much difficulty.

In the latter case, contrary to our expectation, the addition of 1 mol of perbenzoic acid to the sugar will cause the formation of mono-benzoyl triacetyl compound (m.p.=141°—142°). The determination of the constitution of the addition compound established it as 1-benzoyl 2, 4, 6-triacetyl glucose (II), by the study of its chemical reactions (1) it yields neither hydrazone nor semicarbazide, (2) the benzoyl group in the molecule was very easily removed by treating with dilute alkali, (3) when the compound treated with phenyl-hydrazine and acetic acid at room temperature after being hydrolysed with N/5 barium hydroxide solution, gives almost quantitatively phenyl-glucosazone but no crystals of mannose-phenylhydrazone. For the confirmation of the structure, the substance was benzoylated and the product was identified with 1, 2-dibenzoyl 3, 4, 6-triacetyl glucose (m.p.=149°) prepared from 3, 4, 6-triacetyl glucose[1] with benzoyl chloride and pyridine, and their specific rotatory power shown in Table 1 was studied for comparison.

Table 1.

Glucose derivatives	$[\alpha]_D$
1-benzoyl 3, 4, 6-triacetyl glucose	+18.35° (in benzene)
1, 2-dibenzoyl 3, 4, 6-triacetyl glucose	−15.7° (in chloroform)
1-benzoyl 2, 3, 4, 6-tetra-acetyl-glucose	−24.43° (in chloroform)

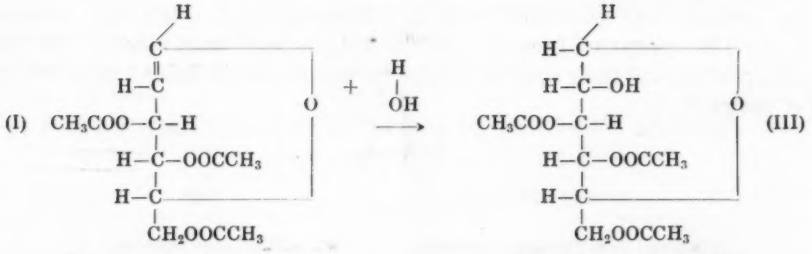
Thus, in the conversion of 1-benzoyl triacetyl β-glucose from triacetyl glucal, the trans-addition of perbenzoic acid to the double bond of the



(1) P. Brigl, *Z. Physiol. Chem.*, **122** (1922), 245.

glucal molecule was assumed to occur, but theoretically the cis-addition is possible in the reaction, by which a new isomeric substance should occur in the reaction product. As a matter of fact, an isomeric substance of m.p.=168° was noticed to arise in the reaction product, but its physical and chemical properties were hardly to be determined owing to the meagreness of the sample.

In the meantime, a third new substance of the formula $C_{12}H_{18}O_8$, m.p.=122°, was isolated from the reaction product, which was supposed to be derived by the addition of H_2O to the triacetyl glucal, this differed in properties from the 2-desoxy glucose prepared by Bergmann[1] from glucal by treating with dilute acid when cold, and did not reduce Fehling's solution and form any oxide, semicarbazone or phenylhydrazone. As a consequence the constitutional formula (III) as 1-desoxy 3,4,6-triacetyl glucose was given for this compound.



Experimental Part

Triacetyl Glucal.[2] Twenty gr. of β-acetobromo glucose[3] (m.p. 87°–88°) which were previously cooled to 0°C., were treated with 40 gr. of zinc powder in 200 c.c. of a 50% acetic acid solution. When all of the β-acetobromo glucose was dissolved, the zinc power was filtered and the filtrate was concentrated to a small volume under reduced pressure to remove acetic acid. The syrup was diluted with 100 c.c. of water, the sugar substance was extracted with ether, neutralized with sodium bicarbonate, washed with water, dried with calcium chloride, and the ether was distilled. The colourless syrup which remained in the flask was changed on standing to white crystals, the yield was about 11.8 gr. This was purified by recry-

(1) *Ber.*, **55** (1922), 158.
(2) E. Fischer, *Ber.*, **47** (1914), 196.
(3) E. Fischer, *Ber.*, **49** (1916), 584.

stallization from absolute alcohol-petroleum ether solution m.p.=54°–55°, $[\alpha]_D^{10}=\frac{-1.03+0.06}{6.298\times 1}\times 100=-15.4°$ in absolute alcohol.

This substance decolourizes bromine water immediately, gives a dark green colour by pine shaving reaction, and reduces Fehling's solution when hot.

Glucal.[1] This substance prepared from triacetyl glucal by following directions of Bergmann & Schotte, was slightly yellow-coloured and is very hygroscopic, melts at 60°–62°, $[\alpha]_D^{3}=\frac{+0.25-0.45}{0.258\times 1}=-7.8°$ in aqueous solution.

Diacetyl Pseudo-glucal. Triacetyl glucal, m.p.=54°–55°, was converted by the method of Bergmann and Ludewig[2] into a colourless syrup of diacetyl pseudo-glucal.

II. Oxidation of Glucal. A cold solution of 3.3 gr. of glucal dissolved in 30 c.c. of absolute methyl alcohol was added with vigorous agitation to an ice-cooled 280 c.c. of perbenzoic acid-chloroform solution (the amount of available oxygen in 1 c.c. of the solution corresponded to 3.40 c.c. of the N/10-$Na_2S_2O_3$ solution) which were prepared freshly by following the directions of J. Levy and R. Lagrave.[3]

Table 2.

Reaction temp.	Reaction time	C.c. of $\frac{N}{10}$ $Na_2S_2O_3$ required for 1 c.c. of the solution
0°	Soon after addition	3.07 c.c.
0°	0.5 h	1.70 ,,
0°	1.0 ,,	1.47 ,,
0°	2.0 ,,	1.47 ,,
9°	24.0 ,,	1.50 ,,

After the reaction was over, the product was treated with some distilled water to extract sugar compounds, and the aqueous solution was neutralized with calcium carbonate, evaporated up to a syrup under reduced pressure, 95% of alcohol was added to remove the salt remaining in the syrup, and

(1) *Ber.*, **54** (1921) 440.
(2) *Ann.*, **434** (1923), 105.
(3) *Bull. soc. chim.*, **37** (1925), 1597.

filtered. When the filtrate was evaporated to a syrup in vacuo, about 3.5 gr. of the colourless syrup were obtained, which gave no Pinoff's reaction. The specific rotation of the syrup was as follows:

$$[\alpha]_D^9=\frac{1.74-0.45}{0.4238\times 1}\times 100=+30.4^\circ \text{ in aqueous solution.}$$

Mannose-phenylhydrazone was prepared in the usual way, melted at 195°–198°, $[\alpha]_D^8=\frac{+0.36-0.15}{0.0802\times 1}\times 100=+26.2^\circ$ in pyridine.

III. Action of Perbenzoic Acid on Triacetyl Glucal. A cold solution of 5.2 gr. of triacetyl glucal dissolved in 50 c.c. of chloroform was added drop by drop to an ice-cooled 140 c.c. of perbenzoic acid-chloroform solution (1 c.c. of the solution corresponding to 4.33 c.c. of the $\frac{N}{10}$ $Na_2S_2O_3$ solution). The progress of the reaction is shown in the following Table 3.

Table 3

Reaction temp.	Reaction time	C.c. of $\frac{1}{10}$ N-$Na_2S_2O_3$ to 1 c.c. of the solution	Blanc test. C.c. of $\frac{1}{10}$ N-$Na_2S_2O_3$ required to 1 c.c. of perbenzoic acid solution
0°	Soon after addition	3.40	4.33
,,	1.0 h ,, ,,	3.40	4.17
,,	2.0 h ,, ,,	3.20	4.33
,,	3.0 h ,, ,,	2.70	4.30
,,	4.0 h ,, ,,	2.65	4.33
9°	24.0 h ,, ,,	1.65	4.33
,,	48.0 h ,, ,,	1.20	4.15
,,	72.0 h ,, ,,	1.00	4.15
,,	96.0 h ,, ,,	0.90	4.15

After about four days, the theoretical amount of oxygen in the sugar solution having been consumed, the reaction product was neutralized with a sodium carbonate solution, washed with water, dried with anhydrous sodium sulphate, and concentrated to a small volume under diminished pressure to remove the chloroform and a colourless syrup was obtained, which changed to crystals on being left standing in an ice box, and weighed ca. 5 gr.

1-Benzoyl 3,4,6-Triacetyl Glucose. The crystals above mentioned were treated with absolute alcohol and 2.5 gr. of the glucose derivative were isolated from the solution. When recrystallized from hot absolute alcohol, it melts at 141°–142°, is soluble in ether and chloroform, slightly soluble in absolute alcohol, but insoluble in water.

The rotatory power in benzene solution shows $[\alpha]_D^8 = +18.35°$ The analytical results are as follows:

Anal. Subst.=0·1148; CO_2=0.2322; H_2O=0.0554 gr. Found: C=55.17; H=5.36%. Calc. for $C_{19}H_{22}O_{10}$: C=55.6; H=5.3%.

Cryoscopy. Subst.=0.2876, glacial acetic acid=15.1050 gr.; depression=0.179°. Mol. wt., found: 414. Subst.=0·4240, glacial acetic acid=15.9090 gr.; depression=0.249°. Mol. wt., found: 417. Mol. wt., calc. for $C_{19}H_{22}O_{10}$: 410.

It reduces Fehling's solution when hot, oxidises Schiff's reagent, and gives the silver mirror with ammoniacal silver nitrate solution, but does not show any mutarotation. When the alcohol solution was heated with dilute alkali, the liberation of the benzoic acid ethyl ester was recognized by its characteristic odour.

The acyl value of the substance was estimated by an N/14 HCl solution after hydrolysing with N/5.32 barium hydroxide solution, and the results agree with the theoretical value for one benzoyl group and three acetyl groups in the molecule.

A. *Hydrolysis of 1-Benzoyl 3,4,6-Triacetyl Glucose.* 1.7 gr. of 1-benzoyl 3,4,6, triacetyl glucose were dissolved in hot alcohol, and 340 c.c. of N/5 barium hydroxide were added, and let stand at room temperature over night. After the solution was neutralized with 1–N sulphuric acid, filtered and concentrated to a small volume under diminished pressure, acidified with some 1–N sulphuric acid, and benzoic acid was extracted with ether. Benzoic acid isolated was found to melt at 122° and confirmed to be so by elementary analysis and study of chemical properties. The yield was 0.5 gr.

Glucosazone. The acidic solution separated from benzoic acid was neutralized with dilute barium hydroxide solution, filtered off the barium sulphate and the filtrate evaporated to a small volume under reduced pressure. Then a mixture of phenylhydrazine and acetic acid, was added to the syrup and heated, and the osazone of m.p. 206° was obtained. The analysis of the substance gave N=15.52%, theory for $C_{18}H_{22}O_4N_4$, N=15.64%.

B. *1-Benzoyl 2,3,4,6-Tetra-acetyl Glucose.* 1.4 gr. of acetic acid anhydride was added to a solution of 0.7 gr. of 1-benzoyl 3,4,6-triacetyl glucose

in 2.1 gr. of pyridine, the mixture was poured into ice water, whereon white crystals (ca. 0.7 gr.) were precipitated, and the substance purified from hot absolute alcohol solution, melts at 145°–146°.

This substance is soluble in chloroform, acetone, benzene, ether, slightly soluble in alcohol, petroleum ether, but insoluble in water.

Rotatory power in chloroform: $[\alpha]_D^4 = \frac{-0.45-0.12}{0.2158\times 1}\times 100 = -26.43°$.

Anal. Subst.=0.1081; CO_2=0.2192; H_2O=0.0538 gr. Found: C=55.30; H=5.53%.

Calc. for $C_{21}H_{24}O_{11}$: C=55·8; H=5.3%.

Molecular weight by Rast's method shows 444, theory 452 for $C_{21}H_{24}O_{11}$.

C. *1,2-Dibenzoyl 3,4,6-Triacetyl Glucose.* 1.0 gr. of benzoyl chloride was added to a solution of 0.6 gr. of 1-benzoyl 3,4,6 triacetyl glucose dissolved in 2.5 gr. of pyridine, and the mixture was left to stand at room temperature for one day, and then poured into ice water, whereon an oily substance was obtained, which solidified gradually. It was dissolved in chloroform, treated with sodium bicarbonate solution, washed with water, dried with anhydrous sodium sulphate, and the chloroform distilled off. The benzoyl acetyl glucose thus obtained, was purified in white crystals having a melting-point of 149°. It is soluble in chloroform, acetone, ether; slightly soluble in alcohol, petroleum ether; but insoluble in water.

$[\alpha]_D^5 = \frac{-0.20-0.12}{0.2031\times 1}\times 100 = -15.7°$ (in chloroform)

Anal. Subst.=0.1069; CO_2=0.2364; H_2O=0.0509 gr. Found: C=60.31; H=5.27%.
Calc. for $C_{26}H_{26}O_{11}$: C=60·7; H=5.06%.

The molecular weight by Rast's method being 506, agrees with the calculated value, 514 for $C_{26}H_{26}O_{11}$

For the preparation of 1, 2-dibenzoyl 3, 4, 6-triacetyl glucose, 1, 2-anhydro 3, 4, 6-triacetyl glucose (m. p.=56°–57°, $[\alpha]_D^{29}$=+106.8° in benzene) prepared by the method by Brigl[1] was used; 2 gr. of the substance dissolved in 100 c.c. of water at room temperature and dried in a vacuum desiccator to a syrup to form 3, 4, 6-triacetyl glucose,[2] which dissolved in 3.0 gr. of pyridine, and 1.5 gr. of benzoyl chloride were added, and the mixture was left to stand for two days, then poured into ice water, whereon an oily substance was obtained, separated, and dissolved in chloroform, treated with sodium bicarbonate solution, washed with water and dried with anhydrous sodium sulphate. By distilling off the chloroform, there were

(1) Loc. cit.
(2) P. Brigl., loc. cit.

obtained white crystals of a melting-point of 149°. The properties of the substance agree well with those of 1, 2-dibenzoyl 3, 4, 6-triacetyl glucose.

$$[\alpha]_D^8 = \frac{-0.12-0.13}{0.1402\times 1}\times 100 = -17.8^\circ \text{ (in chloroform)}$$

Anal. Subst.=0.1011; CO_2=0.2239; H_2O=0.0478 gr. Found: C=60.40; H=5.25%. Calc. for $C_{26}H_{26}O_{11}$: C=60.7; H=5.06%.

The molecular weight by Rast's method gave 507, ($C_{26}H_{26}O_{11}$=514).

1-Desoxy 3, 4, 6-Triacetyl Glucose. The filtrate separated from the crystals of 1-benzoyl 3, 4, 6-triacetyl glucose was poured drop by drop into ice water, whereon a white substance (A) was precipitated, weighing ca. 10 gr., on this being filtered, and the filtrate was concentrated to a syrup under reduced pressure at ordinary temperature, a white semi-solid substance (B) was yielded (ca. 0.6 gr.); this was filtered, and the final filtrate again concentrated to a slightly yellow-coloured syrup. This residue was changed to crystals on being left standing in a ice box, the yield being about 1.4 gr. By recrystallisation from hot 94% alcohol, it melted at 122°. It is soluble in hot ethyl alcohol, acetic acid and water, slightly soluble in benzene and ether, but it does not reduce Fehling's solution before and after treatment with dilute sulphuric acid, and does not oxidise Schiff's reagent. Nor does this substance give silver mirror with ammoniacal silver nitrate, nor change with dilute alkali solution.

Rotatory power in chloroform: $[\alpha]_D^7 = \frac{+0.30-0.12}{2.06\times 1}\times 100 = +8.7^\circ$

The dried substance gave the following analytical results:

Anal. Subst.=0.1158; CO_2=0.2112; H_2O=0.0686 gr. Found: C=49.74; H=6.58%. Calc. for $C_{12}H_{18}O_8$: C=49.7; H=6.2%.

Molecular weight by cryoscopic method in glacial acetic acid shows 283 ($C_{12}H_{18}O_8$=290).

Acetyl value was estimated and the results agreed with the triacetyl in the molecule.

The precipitate (A) was found to consist of a mixture of 0.7 gr. 1-benzoyl 3, 4, 6-triacetyl glucose and 0.3 gr. of 1-desoxy 3, 4, 6-triacetyl glucose.

The Isomer of 1-Benzoyl 3, 4, 6-Triacetyl Glucose. The semi-solid substance (B) was obtained in white crystals (ca. 0.15 gr.) m.p.=168° from an absolute alcohol solution. It reduces Fehling's solution when hot, and it's alcohol solution changes into yellow when heated with dilute alkali. The substance dried on a porcelain tile gave the following analytical results:

Anal. Subst.=0.1036; CO_2=0.2094; H_2O=0.0565 gr. Found: C=55.13; H=6.06%. Calc. for $C_{19}H_{22}O_{10}$: C=55.6; H=5.3%.

The molecular weight by Rast's method gave 401.

From the analytical results and also the chemical properties, it was ascertained to be an isomer of 1-benzoyl 3, 4, 6-triacetyl glucose.

Acetyl Sugar. From the filtrate separated from 1-desoxy 3, 4, 6-triacetyl glucose, 0.4 gr. of the syrup were obtained, which gradually changed to needle crystals. This substance is soluble in alcohol, ether and water, but insoluble in petroleum ether. It reduces Fehling's solution when hot.

$$[\alpha]_D^9 = \frac{+1.07-0.14}{0.2106\times 1}\times 100 = +44.15^\circ \quad \text{(in chloroform)}$$

IV. Action of Perbenzoic Acid on Diacetyl Pseudo-glucal. A cold solution of 7.5 gr. of diacetyl pseudo-glucal dissolved in 50 c.c. of chloroform were added to an ice-cooled solution of 220 c.c. of perbenzoic acid-chloroform (the amount of active oxygen in 1 c.c. of the solution corresponded to 3.80 c.c. of the N/10 $Na_2S_2O_3$ solution). The progress of reaction was very slow, as shown in the following Table 4, and almost all of the substance remained unchanged.

Table 4.

Reaction temp.	Reaction time	Number of c.c. of $\frac{N}{10}$ $Na_2S_2O_3$ solution required for 1 c.c. of the solution
0°	Soon after addition	3.14
0°	0.5 h ,, ,,	3.14
0°	3.5 h ,, ,,	3.06
8°	24.0 h ,, ,,	2.85
8°	48.0 h ,, ,,	2.85
9°	72.0 h ,, ,,	2.90

In conclusion, the present writer wishes to express his hearty thanks to Prof. S. Komatsu for his masterly guidance and also to Prof. Nodzu for his invaluable advice.

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SINOMENINE AND DISINOMENINE. XVII. ON METHYL-SINOMENININONE.

By Kakuji GOTO, Taro NAMBO and Reikichi INABA.

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One of the authors (K.G.) has reported that the action of $2n$ hydrochloric acid on sinomenine[1] at 100°C. gives rise to the formation of sinomenine hydrate (II)[1] and the same treatment of 1-Bromo-sinomenine leads to the formation of 1-Bromo-sinomeninone (III)[2] The difference in the behaviour of those two very similarly constituted bases might be due to the negativity of bromine atom in the latter. So, it was desirable to expose methyl-sinomenine to the same treatment in order to see whether it gives hydrate or diketone.

The experiment revealed that methyl-sinomenine was simply hydrolysed on its enol-ether group by the action of $2n$ hydrochloric acid at 100°C. and gave methyl-sinomeninone (III) in a yield about 70%. The hydrate was not isolated in this case, too, so it seems that the stage of hydrate was jumped over both in bromo- and methyl-sinomenine in the formation of the respective diketones.

By the action of concentrated ammonia, sinomenine hydrate was turned into a bimolecular bis-demethyl-sinomenyliden,[3] and bromo-sinomeninone into a mono-molecular bromo-sinomeninone imine,[4] but the methyl-sinomeninone was turned into an amorphous substance by the same reagent, and no base corresponding to one of the above substances could not be isolated.

By the reduction with $Pd+H_2$, methyl-sinomeninone absorbed two atoms of hydrogen and gave a new base $C_{19}H_{25}NO_4$, which formed a monoxime, showing that one of the two ketone groups was reduced to the alcohol group. This base should be called dihydro-methyl-sinomeninone (V).

It is interesting to examine which of the two ketone groups of the methyl-sinomeninone was reduced in this case. The clew may be obtained from the methylation of α- and β-demethoxy-sinomenine hydrate. But,

(1) Goto and Sudzuki, this Bulletin, **4** (1929), 271.
(2) Goto and Nambo, this Bulletin, **5** (1930), 73.
(3) Loc. cit.
(4) Loc. cit.

unfortunately the reaction products of these two substances with diazomethane became syruppy and no conclusion could be obtained in this trial.

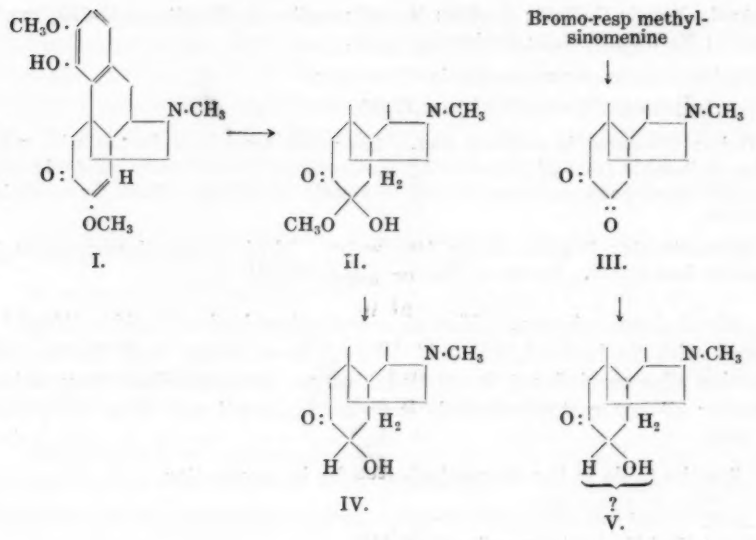
Then we tried to reduce catalytically bromo-sinomeninone and compared the reduced base with brominated α-demethoxy-sinomenine hydrate. From the mode of preparation, it is almost clear that α-demethoxy-sinomenine hydrate has its ketone group on C_6 and its secondary alcohol group on C_7 (IV). If, by the reduction of bromo-sinomeninone, the identical base was obtained as with the bromination of α-demethoxy-sinomenine hydrate, we may assume with some certainty that in the reduction of bromo-sinomeninone the ketone group situated in C_7 has been reduced. The result was affirmative. The substances prepared in these two different ways melted both at 231°C. as well as the admixture.

From these results, we should like to assume that in dihydromethyl-sinomeninone, the ketone group situated at C_7 is also reduced, though an exact proof is still lacking.

In these experiments, the methylation of sinomenine was again studied in a more detailed way and the yield was improved. Benzoylation of sinomenine was carried out with benzoyl chloride in pyridine solution with better yield. The detailed conditions are given in the experimental part.

Table 1.

	Methyl-sinomenine	Methyl-sinomeninone	Dihydromethyl-sinomeninone	Benzoyl-sinomenine
Yield	30–35%	70%	80%	40%
Solubility in methyl alcohol	12–52%	4.47%	—	—
M.p.	179°	188°	127° (dried) 108° (air dry)	225°
$[\alpha]_D$	−29.61°	+18.65°	+71.05°	+85.03°
Methoxyls	3	2	2	2
M.p. of oxim	139°	213°	117°	249°
M.p. of iodomethylate	157° (dec.)	225°~227°	248°	237°
$FeCl_3$-reaction	no	no	no	no
Diazo-reaction	20,000	10,000	almost no	appears slowly
$K_3Fe(CN)_6$-reaction	no	no	no	no
Formaline-sulphuric acid	yellow→green	green→bordeau	yellow	yellow



Experimental.

Note on Preparation of Methyl Sinomenine with Nascent Diazomethane. A better yield (30–35%) was attained in using methyl alcoholic solution of sodium hydroxide in an almost calculated quantity. Purification is easy, taking advantage of the facts that methyl-sinomenine is more easily soluble in ether than sinomenine itself and also that the hydrochloride of the former is less soluble in water (3.7%) than that of the latter (4.5%). Twice recrystallised from water, the methyl-sinomenine gives no ferric chloride reaction.

With diazomethane in ethereal solution, no better yield was obtained than in the above methcd.

The phenol-hydroxyl of sinomenine is, however, very easily methylated with dimethyl sulphate and caustic alkali, the fact being shown by the disappearance of the diazo-coupling faculty. But, the greater part of the sinomenine is splitted in its $N-C_9$ linking by this treatment.

The phenol-hydroxyl is also methylated by methyl-iodide and alkali in methyl alcoholic solution. But, the seperation of methyl-sinomenine iodomethylate from sinomenine iodomethylate gives some difficulties.

As for the properties, see the first column of the table in the theoretical part.

Anal. Found: C=70.03; H=7.49; N=4.03; methoxyl=26.51%. $C_{20}H_{25}NO_4$ requires C=69.97; H=7.29; N=4.08; Methoxyl=27.11%.

Optical rotatory power measured in chloroform:

$[\alpha]_D^{14}=(10\times0.52)\div(0.3515\times0.5)=-29.61°$

Oxim: Prepared in ordinary way. Amorphous precipitate formed with sodium carbonate becomes stout prisms, when left stand overnight under water added with ether. M.p. 139° (decomposes between 150–160°). Found: N=7.66%. Calc. for monoxime: N=7.82%.

Iodomethylate: Recrystallisable from water. Dired crystals decomposes at 151° (sintering from 141°C). Found: I=26.04% Calc. I=26.18%.

Metyl-sinomeninone. Methyl-sinomenine hydrochloride (10 gr.) is heated with 2*n* hydrochloric acid (50 c.c.) on a steam bath for an hour. Isolation was carried out in an usual way. Recrystallised from a large quantity of hot methyl alcohol, it forms large prisms. M.p. 188°. Yield ca. 70%.

See the table in the theoretical part for its properties.

Anal. Found: C=69.21; H=7.00; N=4.20; methoxyl=19.11%. $C_{19}H_{23}NO_4$ requires C=69.30; H=6.99: N=4.25; methoxyl=18.88%.

Sp. rotatory power, measured in chloroform: $[\alpha]_D^{14}=(10\times0.30)\div(0.5\times0.3216)=+18.65°$

Oxim: Prepared and crystallised in the same way as with methyl-sinomenine. M.p. is very unsharp, sintering above 100°, melting at 170° and decomposing completely at 213°. Found: N=11.74% Calc. for dioxime: N=11.70%.

Iodomethylate. Stout prisms, decomposing at 225~227° with yellow colour. Found: I=26.51% Calc. I=26.96%.

Dihydromethyl-sinomeninone. Methyl-sinomeninone (1.9 gr.) was reduced catalytically with palladinised charcoal and hydrogen gas in weakly acidic solution. Absorption of H_2 amounted to 50 c.c. (ca. 1 mol.) in 50 minutes. Isolation was carried out in an usual manner. It crystallises out in long prisms from the 33% methyl alcohol solution, saturated with ether. M.p. 108° (air-dry) or 128° (dried over P_2O_5 in vacuum). Yield ca. 1.5 gr. (80%).

Properties were given in the table in the theoretical part.

Anal. Found: C=68.61; H=7.62; N=4.41, 4.20; methoxyl=18.93%. $C_{19}H_{25}NO_4$ requires: C=68.84; H=7.60; N=4.25; methoxyl=18.73%.

Sp. rotatory power, measured in chloroform: $[\alpha]_D^{15}=(10\times0.81)\div(0.2280\times0.5)=+71.05°$

Oxim: Crystallised from methyl alcohol. M.p. 177°. Yield ca. 40%. Found: N=7.67%. Calc. for monoxime: N=8.09%.

Iodomethylate. Long needles from methyl alcohol. M.p. 248° (dec.). Yield good. Found: I=26.35%. Calc.: I=26.85%.

Benzoyl-sinomenine. Sinomenine (2.5 gr.) in a pyridine solution (12.5 gr.) was added with benzoyl chloride (3 c.c.) at 10°C. After half an hour, the pyridine solution was poured into the mixture of ice (100 gr.) and saturated ammonium carbonate solution. After standing two days, when the first appeared oil drops changed into fine solid, the latter was collected and recrystallised from the mixture of benzol and ether. M.p. 224°. Yield ca. 35%. This preparation is far more convenient than that formerly given.

Anal. Found: C=71.81; H=5.77; N=3.23; methoxyl=14.21%. Calc. for $C_{26}H_{27}NO_5$= 433: C=72.05; H=6.23; N=3.23; methoxyl=14.31%.

Sp. rotatory power measured in chloroform: $[\alpha]_D^4=(10\times2.5)\div(0.294\times1)=-85.03°$

Iodomethylate: hairy crystalls from water. M.p. 237° (dec.). Found: I=22.14%. Calc.: I=22.08%.

Oxim: Prepared in ordinary way and extracted with chloroform. Prisms from methyl alcohol. M.p. 249° (dec.) Found: N=5.99%. Calc. for monoxime: N=6.25%.

Bromination of α-desmethoxyl-sinomenine hydrate.[1] The condition of bromination is the same as with sinomenine.[2] From a large quantity of alcohol, it forms long prisms, which melt at 231°. Yield about 50%.

Anal. Found: C=54.79; H=5.43; N=5.5. $C_{18}H_{22}BrNO_4$ requires: C=54.55; H=5.55; N=3.53; Br=20.20%.

Oxim: M.p. 147°. Found: N=6.54%. Calc.: N=6.81%.

Catalytic Reduction of Bromo-sinomeninone. Bromo-sinomeninone (2 gr.) was catalytically reduced with palladinised charcoal and molecular hydrogen. When the calculated quantity of hydrogen was absorbed the reduced base was isolated in the usual way. Yield good. It melted at 231° and the admixture with the above described specimen did not lower the melting point.

Errata.—To the constitution formula II of p. 94, this Volume, the following corrections must be given. The methoxyl on $C_{(7)}$ must be replaced by H. The—H on $C_{(8)}$ must be replaced by H and one free bond. Thus, the formula is brought in harmony with the facts and its name 1, 1′-dibromo-bis-[8, 8′]-demethoxy-dihydro-sinomenine.

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(1) Loc. cit., p. 275.
(2) Goto and Nambo, this Bulletin, **5** (1930), 167.